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Seed Physiological Potential of *Capsicum annuum* var. *glabriusculum* Genotypes and Their Answers to Pre-Germination Treatments

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Abstract: Piquin pepper (*Capsicum annuum* var. *glabriusculum*) is an important species that supports the economy of rural households; it is part of Mexican gastronomy and it is a highly valuable phytogenetic resource. There has been recent interest in domesticating and exploiting piquin pepper commercially, which has been limited until now due to the low germination rate, and this work had the purpose of promoting germination and determining the physiological capacity of genotypes. Ten piquin pepper genotypes from different geographical origins in Mexico were submitted to 11 pre-germination treatments. A completely randomized experimental design was carried out with arrangement in split-plot. The large plot had the treatments and the small plot had the genotypes. The results showed differences ($p < 0.01$) among treatments, genotypes, and treatment–genotype interaction. On one hand, treatments gibberellic acid (GA) and mechanical scarification + gibberellic acid (MSGA) increased the physiological potential of genotypes, reaching the highest values of germination speed (GS), germination index (IG) and germination percentage (GP); as well as the lowest values of dead seeds (DS) and hard Seeds (HS). In turn, the genotypes that presented the same condition were G8, G7, and G10. Regarding the interaction, each variable had a different condition. In conclusion, we can increase the physiological potential and solve the dormancy of piquin pepper seed by applying gibberellic acid. Likewise, the best genotypes were G8 and G10.

Keywords: piquin pepper; dormancy; physiology; Treatment-Genotype interaction; biplot

1. Introduction

Piquin pepper (*Capsicum annuum* var. *glabriusculum*) has several names, including "chiltepín", "chile de monte", and "chile silvestre" (wild pepper), among others [1]. The distribution of this species reaches Colombia, Central America, Mexico, and the South of United States [2]. In Mexico, it is widespread from Sonora to Chiapas and from Tamaulipas to Yucatan and Quintana Roo, showing great environmental adaptability, probably resulting from its genetic diversity that allows it to adapt itself to different environments [3,4]. The importance of piquin pepper lies in the fact that it is the main source of income for many people and households in rural areas, who make their living from picking of the fruit of wild populations. This hot pepper is preferred over Jalapeño and Serrano

hot peppers in the market, although its value can be up to 40 times more expensive [5,6]. The great acceptance of piquin pepper by consumers is due to its pleasant flavor, and even when it is very spicy (50,000–100,000 SHU), that sensation disappears quickly from the mouth and does not irritate the digestive system [7]. Furthermore, it is a highly valuable resource in breeding programs, since it is considered the ancestor of all *Capsicum annuum* [8,9]. At present, there is great interest in domesticating this species and establishing it as a commercial crop. It is known that piquin pepper seeds have a natural dormancy mechanism that acts as a potential blockage to complete germination [3]. This mechanism has helped the species to survive under adverse climate conditions and avoid competing for light, water, and nutrients [10,11]. From an agronomic management standpoint, this is the main problem of piquin pepper; the dormancy mechanism results in low and uneven germination at planting [7]. Therefore, the purpose of this research work was to determine the effect of pre-germination treatments on the germination traits of different piquin pepper genotypes and to assess their physiological capacity.

2. Materials and Methods

2.1. Location of the Experimental Site

The experiment took place in the Seed Test Laboratory of the Training and Development Seed Technology Center, "Centro de Capacitación y Desarrollo de Tecnología de Semillas" (CCDTS) from the Plant Breeding Division of "Universidad Autónoma Agraria Antonio Narro" (UAAAN), located in Buenavista, Saltillo, Coahuila, Mexico.

2.2. Plant Material

This research work included ten piquin pepper genotypes from different geographical origins in Mexico provided by the National Institute of Forestry, Agricultural and Livestock Research, "Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias" (INIFAP), "Las Huastecas" Experimental field (Table 1). The mother plants of each genotype were kept in a controlled greenhouse environment, so they had the same conditions. For the obtaining of the seed, ripe fruits were collected, which were allowed to dry under ambient temperature conditions, were placed in paper bags with their identification, and then stored for 3 months at a temperature of 17 °C. Subsequently the seed of the fruits was extracted, at the time it was required to use it.

Table 1. Identification and origin of 10 piquin pepper genotypes.

ID	Locality	Municipality	State	Geographical Coordinates
G1	Estación Álamo	Villaldama	Nuevo León	26°23′51″ N, 100°23′42″ W
G2	Ej. Potrero de Zamora	Aramberri	Nuevo León	24°02′20″ N, 99°55′15″ W
G3	Ej. Lázaro Cárdenas	Burgos	Tamaulipas	24°56′56″ N, 98°47′59″ W
G4	Barranco Azul	San Carlos	Tamaulipas	24°24′01″ N, 99°06′50″ W
G5	Palo Blanco	Castaños	Coahuila	26°46′02″ N, 101°30′01″ W
G6	Colatlán	Ixhuatlán de Madero	Veracruz	20°49′04″ N, 98°05′45″ W
G7	Tiopancahuatl	Ixhuatlán de Madero	Veracruz	20°41′06″ N, 98°00′44″ W
G8	Colatlán (Nursery)	Ixhuatlán de Madero	Veracruz	20°49′04″ N, 98°05′45″ W
G9	El Rincón	Linares	Nuevo León	24°53′40″ N, 99°28′13″ W
G10	La Laborcilla	Rioverde	San Luis Potosí	21°51′37″ N, 99°54′40″ W

2.3. Treatment of Seeds

Eleven pre-germination treatments showed in Table 2 were used in order to evaluate the germination response in piquin pepper seeds. The treatments were selected according to previous works, due to their effect on the seed [3,12–18]. The distilled water was used as a solvent to obtain the desired concentration. In each treatment the seed was submerged for the indicated time, except for the mechanical scarification, which consisted of sanding the seed. After submitting the seed to each

treatment, a rinsing with distilled water was carried out. Three replicates were performed and 20 seeds per replicate of each genotype were used.

The seeds were disinfected with sodium hypochlorite at 1% during 30 s, before rinsing them with running water under the faucet, followed by a final rinse with distilled water. The seeds were spread over absorbing paper and were left to dry for 15 min. The corresponding pre-germination treatments were applied afterwards (Table 2). Petri dishes (100 mm × 15 mm) lined with sterilized filter paper were used, moistened with a solution of distilled water and fungicide. Each week two types of fungicides were rotated to prevent and control the development of pathogenic fungi, namely Captan 50 (Captan) and Tecto 60 (Thiabendazole). Both at a concentration of 1 g L^{-1}, were applied by spray at the time the filter paper required moisture. This was done throughout the duration of the assay. The seeds were planted in the dishes by distributing them in circles, a small and a bigger circle, to facilitate the evaluation of the variables. Afterwards, the Petri dishes were put into the germination chamber LAB-LINE at 25 °C, with 12 h of light and 12 h of darkness.

Table 2. Treatments used to evaluate the germination response of piquin pepper seeds.

ID	Treatment	Application	Time
HP	Hydrogen peroxide	3%	24 h
GA	Gibberellic acid	5000 ppm	24 h
AV	Agromil-V®	2% *v/v*	24 h
HC	Hydrochloric acid	10%	30 min
KN	Potassium nitrate	3%	168 h
HW	Hot water	At 83 °C let cool naturally	24 h
MS	Mechanical scarification	Sanding softly with 1500 grit sandpaper	30 s
HWGA	Hot water + Gibberellic acid		24 h per tratament
HPGA	Hydrogen peroxide + Gibberellic acid		24 h per tratament
HCGA	Hydrochloric acid + Gibberellic acid		1st tratament 30 min and 2nd tratament 24 h
MSGA	Mechanical scarification + Gibberellic acid		2nd tratament 24 h
WIT	Witness	Direct seeding	

2.4. Assessed Seed Germination Traits (Variables)

Germination percentage (GP): it was defined as the relationship among the number of germinated seeds that had all their essential structures and the number of planted seeds, using the following formula: GP = (NGS/TNS), where NGS is the number of germinated seeds and TNS is the total number of planted seeds. Germination index (GI): is the time of germination based on the germination capacity, GI = Σ(*niti*)/TNS, where *ni*, is the number of seeds germinated in day *i* and *ti* is the number of days after planting. Germination speed (GS): is the number of seeds germinated in relation to the time of germination: GS = Σ(*ni*)/*t*, where *t* is the germination time measured in days, from planting until the germination of the last seed. Abnormal seedlings (AP): those seedlings that lacked one or more of their essential seedling structures were counted. Dead seeds (DS): The counting included all those seeds that were soft; they absorbed water, but they did not produce any seedlings. Hard seeds (HS): all those seeds that remained waterproof at the end of the assay were counted, since they did not absorb water. The results of these last three variables were expressed in percentages.

2.5. Statistical Analysis

A randomized experimental design was used with arrangement in split-plot. The large plot had the treatments and the small plot had the genotypes. In order to increase the data normality before the analysis, the percentages were converted into square root values.

The data was analyzed using a variance analysis in accordance with our design. When the differences were significant, Tukey's multiple comparison test was performed, and Biplot graphics

were used for the interactions. Furthermore, the relationship among the variables was studied using Spearman correlation coefficient and the genotypes similarity was determined by a dendrogram using the UPGMA technique. All analyses were performed using the TukeyC, agricolae, and corrplot packages of the statistical software R version 3.5 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

In the variance analysis of the physiological tests, highly significant differences ($p < 0.01$) were found in the sources of variation treatments, genotypes and treatment–genotype interaction (Table 3). This could be due to the different genetic constitution of the genotypes studied and the particular effect of the treatments.

Table 3. Mean squares of the analysis of variance of physiological variables.

SV	DF	GS		GI		GP		AP		DS		HS	
Treat	11	1.285	**	9.590	**	0.129	**	0.079	**	0.045	**	0.150	**
Error (a)	24	0.004		0.134		0.002		0.002		0.004		0.004	
Gen	9	0.331	**	5.149	**	0.062	**	0.018	**	0.074	**	0.029	**
Treat × Gen	99	0.030	**	0.442	**	0.007	**	0.005	**	0.007	**	0.009	**
Error (b)	216	0.006		0.115		0.002		0.002		0.002		0.003	
CV (a)		4.89		18.68		4.34		4.49		5.13		5.200	
CV (b)		6.12		17.35		4.27		3.83		4.14		4.500	

** Significant at probability levels ≤ 0.01, SV = Sources of Variation, DF = Degrees of Freedom, GS = Germination speed, GI = Germination index, GP = Germination percentage, AP = Abnormal seedlings, DS = Dead seeds, HS = Hard seeds, Treat = Treatments, Gen = Genotypes, % CV = Coefficient of Variation.

3.1. Effects of Pre-Germination Treatments

According to the mean comparison test (Table 4), the gibberellic acid treatment and its combination with mechanical scarification (sanding) showed superior results, with increases in GS (77%), GI (52%), GP (69%), and a decrease in DS (35%) and HS (74%), in comparison with the rest of the treatments.

Table 4. Comparison of Tukey's means ($p > 0.05$) for pre-germinative treatments in physiological variables.

Treat	GS		GI		GP		AP		DS		HS	
AV	0.41	c	3.35	cd	15.17	bc	9.83	cde	30.67	cde	44.33	abc
GA	1.52	a	6.19	a	40.00	a	26.67	ab	21.83	e	11.17	d
HC	0.46	c	4.54	bc	0.00	e	30.67	a	34.83	bcde	34.5	c
HCGA	1.22	b	5.79	ab	37.33	a	19.67	bc	26.5	de	16.5	d
HP	0.11	de	1.53	ef	5.17	de	4.00	de	39.83	bc	51.00	ab
HPGA	0.40	c	3.61	cd	13.83	bcd	11.83	cd	36.17	bcd	38.17	bc
HW	0.02	e	0.20	fg	1.00	e	0.33	e	53.67	a	45.00	abc
HWGA	0.00	e	0.00	g	0.00	e	0.00	e	45.00	ab	55.33	a
KN	0.18	d	2.48	de	8.67	cde	5.17	de	31.5	cde	54.67	a
MS	0.19	d	2.50	de	9.67	cde	4.33	de	34.33	bcde	51.67	ab
MSGA	1.46	a	6.01	ab	34.17	a	29.5	ab	24.67	de	11.67	d
WIT	0.38	c	5.19	ab	23.33	b	6.67	de	26.33	de	43.67	abc

Means with the same letter are not significantly different, Treat = Treatment, GS = Germination speed, GI = Germination index, GP = Germination percentage, AP = Abnormal seedlings, DS = Dead seeds, HS = Hard seeds, AV = Agromil-V®, GA = Gibberellic acid, HC = Hydrochloric acid, HCGA = Hydrochloric acid + Gibberellic acid, HP = Hydrogen peroxide, HPGA = Hydrogen peroxide + Gibberellic acid, HW = Hot water, HWGA = Hot water + Gibberellic acid, KN = Potassium nitrate, MS = Mechanical scarification, MSGA = Mechanical scarification + Gibberellic acid, WIT = Witness.

Hot water and its combined with gibberellic acid affected in a negative way the germination variables, reducing GS by 97%, GI by 97%, and GP by 96%; on the other hand, it increased the percentages of DS (27%) and HS (13%) as compared to the average of the rest of the treatments. It also

showed low AP values, which, despite being a positive aspect, was influenced by low germination, in this case.

Hydrogen peroxide and its combination with gibberellic acid, besides potassium nitrate, showed lower results than the witness; obtaining 40, 51, and 61% less GS, GI, and GP respectively, besides the increases of 5, 27 and 9% in AP, DS, and HS.

The treatments to break physiological dormancy presented better results than the treatments that were used to break physical dormancy, which is an indication that there are inhibiting physiological mechanisms that impair germination.

3.2. Physiological Response of Genotypes

With regards to the genotypes (Table 5), the results have shown that G8, G7, and G10 had the ideal traits, by presenting increases in the germination variables (51% of GS, 53% of GI, and 66% of GP), besides having the lowest values of DS and HS (48% and 11% less) as compared with the rest of genotypes. Likewise, the variation of results might be due to the different environmental conditions to which each genotype had to adapt and therefore the physiological needs were different.

G1 and G5 showed a deficient answer by giving very low values in the desirable traits and very high values in the undesirable traits, with the exception of AP. This might be due to the conditions in which they normally develop, causing different needs.

Table 5. Comparison of Tukey's means ($p > 0.05$) for genotypes in physiological variables.

Genotype	GS		GI		GP		AP		DS		HS	
G1	0.29	e	1.73	fg	5.14	de	9.72	c	38.06	abc	47.08	a
G2	0.59	cd	3.57	cd	18.19	b	11.67	bc	30.83	cde	39.03	abc
G3	0.54	d	3.34	cde	14.31	bc	13.19	bc	45.14	a	27.36	c
G4	0.50	d	3.01	def	12.36	bcd	13.06	bc	33.47	bcd	41.11	a
G5	0.28	e	2.19	efg	6.11	cde	10.42	c	44.72	ab	38.75	abc
G6	0.47	d	3.16	de	15.97	b	9.31	c	38.75	abc	35.97	abc
G7	0.67	c	4.63	bc	27.08	a	9.58	c	23.61	def	39.72	ab
G8	1.01	a	5.46	ab	27.64	a	22.36	a	21.11	ef	29.17	bc
G9	0.15	f	1.05	g	3.06	e	5.14	c	44.86	ab	46.94	a
G10	0.80	b	6.35	a	27.08	a	19.44	ab	17.22	f	36.25	abc

Means with the same letter are not significantly different, GS = Germination speed, GI = Germination index, GP = Germination percentage, AP = Abnormal seedlings, DS = Dead seeds, HS = Hard seeds.

3.3. Specific Response of Treatment by Genotype

According to the main component analysis (Figure 1), the two first components contributed to explaining from 53.8 to 72% out of the total variation. PC1 explained the greatest variation from 33.7 to 56.3%; followed by PC2, with a variation of 20.1 to 26.2%. A different distribution was observed in the genotypes and in the treatments for each variable, which is indicative of a great genetic and physiological diversity of seeds.

Figure 1 GS corresponds to the germination speed, determined that genotype G8 had the best response despite being the least stable, followed by genotypes G2 and G10, that also had positive answers. These results were observed when applying GA treatments and their combinations with MS and HC. On the other hand, genotypes G5 and G9 had very different responses, influenced by negative environments (the one that is not suitable for genotypes to express desirable characteristics, but on the contrary), such as HW, HWGA, HP, and MS. Genotypes G4, G5, G6, and G7 were the most stable. For this variable, genotypes G8 and G9 had the highest contribution of G and/or GE.

Regarding Figure 1 GI, shows that genotypes with the desirable trait were G10 and G8 in HPGA and HC environments, the latter being the most discriminating. In turn, these environments presented negative correlation with HW, HWGA, HCGA, GA, MSGA, MS, and KN. The genotypes with greater

stability were G5, G6, and G7, since they were closer to the origin of PC2 associated to stability of the genotypes.

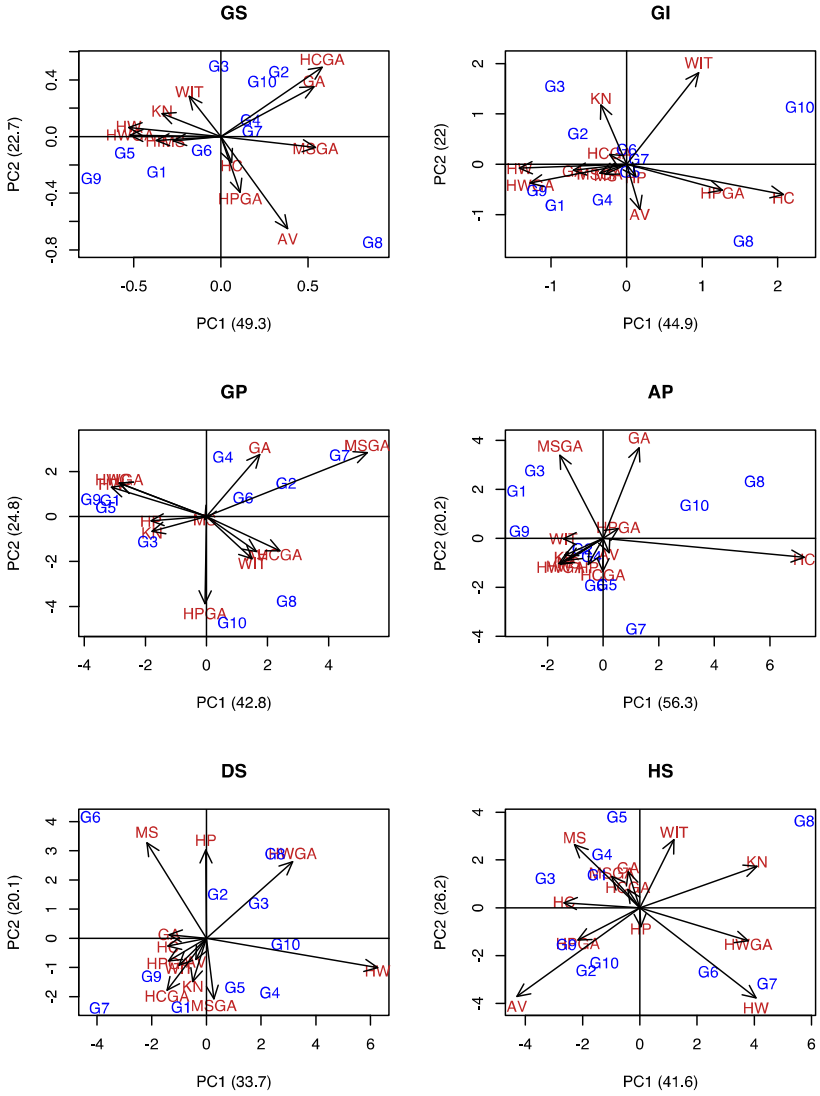

Figure 1. Principal component analysis of physiological variables of interaction Treatment by Genotype, (GS) Germination speed, (GI) Germination index, (GP) Germination percentage, (AP) Abnormal seedlings, (DS) Dead seeds, (HS) Hard seeds.

Figure 1 GP, regarding the percentage of germination, shows that genotypes G2, G7, and G8 obtained the highest values. The first one and the second one behaved well in MSGA, considering that this was the most discriminating and most representative environment for this variable. Genotype G8 showed a higher than average response in HCGA, AV, and WIT environments. It is worthwhile mentioning that most of the treatments containing gibberellic acid were grouped and considered as a favorable environment, with the exception of HWGA that was classified as a negative environment, together with HW and HC. These last three treatments contributed to decrease the values of genotypes G1, G5, and G9, considered among the most stable.

One of the desirable traits was having a low percentage of abnormal plants. Genotypes G1 and G3 in MSGA environment and genotype G9 in WIT complied with this trait. The opposite happened with genotypes G8 and G10 in HC, an environment that besides being the least ideal is the most discriminating one, since it presented the longest vector (Figure 1 AP).

As for the percentage of dead seeds, treatment HW showed a negative correlation with most of the treatments; with the exception of HWGA and MSGA, since they had angles greater than 90° between their vectors. HW was the environment with the highest percentage of dead seeds and it was the most discriminating environment. On the other hand, Genotype G6 showed good adaptation in treatment MS, resulting in lower incidence of dead seeds. Genotype G7 also presented a positive response in HPGA (Figure 1 DS).

Regarding the percentage of hard seeds, Figure 1 HS shows that there are specific interactions of each genotype with every environment. The genotypes showing lower percentage of this trait were G3 with applying HC, as well as G9 applying HPGA and AV. On the other hand, AV and HW were the most discriminating treatments. Furthermore, HW, HWGA, and KN interacted in a negative way with most of the genotypes.

3.4. Association between Physiological Variables

Figure 2 shows the association among the test variables. Germination variables (GI, GS, and GP) were closely related in a positive way, besides being influenced by AP. These variables were negatively associated with DS and HS. These data allow us to forecast the behavior of piquin pepper seed in an indirect way.

Figure 2. Correlation of physiological variables of piquin pepper, (GS) Germination speed, (GI) Germination index, (GP) Germination percentage, (AP) Abnormal seedlings, (DS) Dead seeds, (HS) Hard seeds.

3.5. Similarity of Genotypes

A cluster analysis using UPGMA, gathered the 10 genotypes in three groups, at a distance of 17 units. The first group included three genotypes with the highest seed physiology potential. The second group had three genotypes with intermediate influence. The third group was the largest, with four genotypes of poor performance. These results show variability among genotypes and we believe that most of these groups were formed due to their geographical closeness, leading to similar needs, with the exception of genotype G6 (Figure 3).

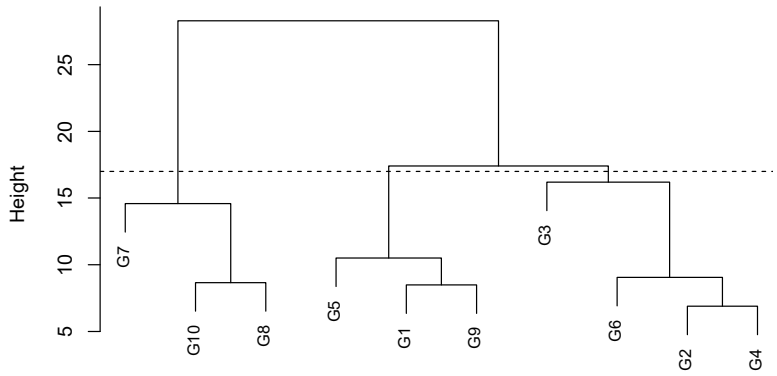

Figure 3. Dendrogram of physiological characteristics of 10 piquin pepper genotypes.

4. Discussion

4.1. Treatment Effect on Seed Physiology

Pre-germination treatments can present specific effects on piquin pepper seeds. Koornef & Bentsink [19] mentioned that the use of gibberellic acid increased the seed germination rates of several species. In this assay, the values were similar to the values reported by García et al. [16], where seed germination and seedling vigor increased after applying this chemical to piquin pepper seeds under greenhouse conditions. Likewise, Née et al. [20] mention that the dormancy-breaking is controlled by various regulators, such as plant hormones and latent proteins. Hot water applications produced negative effects in germination, coinciding with the results of García et al. [16], who reported that the hot water treatment in piquin pepper seeds delays germination and seedling emergence. On the other hand, we differed with the results reported by Cano et al. [3] who reported that hydrogen peroxide and potassium nitrate applied to 16 collections of piquin pepper did not modify the germination percentage, as compared to the witness (without any treatment).

4.2. Variability among Genotypes

Due to the diversity presented by genotypes and their great adaptability to different environments, this species is an important phytogenetic resource for pepper crops' breeding programs [21]. Differences due to genetic diversity of genotypes can provide high phenotypic plasticity and variation of morphological traits [22]. Therefore, the variation of the germination capacity among and within plant species is an adaptation to the particular conditions of local and regional habitats [23]. Within the same context, Hernández-Verdugo et al. [24] found great variation in the germination capacity of wild pepper related populations. This variation allowed the detection of three genotypes with physiological potential.

4.3. Interaction between Genotypes and Treatments

The interpretation of the interactions can be done by means of biplot graphics. Genotypes with PC1 > 0 were identified as having positive value according to the variable studied and their opposites were identified as PC1 < 0 [25,26]. Likewise, the genotypes located farther from the center contributed the most to G and/or GE [27]. On the other hand, the environments with the longest vectors were the most discriminating ones [28]. In turn, the angle formed between the location of genotypes and their environments, has a correlation. The correlation is negative if the angle is obtuse, while an acute angle depicts a positive relation [29,30]. On the other hand, PC2 is related to the stability of the genotypes, being more stable than those genotypes that approached 0 [31]. Concerning the relationship between the genotype and the environment, the closer were the genotypes from the vector, the greater their adaptation to that specific environment [32].

In this assay, three genotypes performed well in combination with GA that promote quick germination [33]. Regarding the GI variable, the HC and HP treatments had a positive effect in two genotypes, simulating the effect of the digestive tract of birds in breaking dormancy in piquin pepper's seeds [15]. In the case of GP, some genotypes required gibberellic acid to promote a positive effect on germination, leading to pre-germination metabolic processes that reduced dormancy drastically. However, in order to reach better results, it was necessary to combine the application of gibberellic acid with treatments that control physical dormancy, such as mechanical scarification (sanding) and hydrochloric acid applications, to improve water uptake and help the emergence of the primary root through the seed coat [15,34]. Negative environments affected those genotypes that were close to the vector, such as hot water, which decreased the GP variable value in three genotypes, and increased the incidence of DS in three other genotypes. In previous works, the same condition has been observed in comparison with other treatments, since temperature is an important factor that affects latency and germination [35,36]. Abnormal seedlings are an undesirable condition that can be caused by environmental factors. In this case, HC promoted an increase of this variable, specifically in two genotypes, by interrupting the germination of the seeds, which could have altered the hormonal metabolites and their regulators [37]. The opposite happened with direct planting (WIT) and seed sanding, where the three genotypes drastically reduced the values of this variable. One of the best-known mechanisms of dormancy is the waterproof seed coat (hence the name "hard seeds"), where dormancy persists until part of the seed coat cracks and allows water to penetrate, giving rise to the imbibition process [38]. The genotypes presented this germination-limiting condition at different levels, but germination improved after the application of hormones like GA and AV. These applications, combined with scarification treatments; either mechanical sanding (MS) or acid applications (HC), can improve that impact. These results confirm that endogenous plant hormones like GA play an important role in seed dormancy release. On one hand, ABA inhibits germination, while on the other hand GA increases germination [39]. Also, the methods to soften artificially waterproof seeds can cause positive changes in germination [40].

4.4. Relationship among Physiological Variables Related to Germination

Knowledge of the existing correlations among the traits of economic importance is a useful tool for indirect selection [41]. The increase in desirable variables (GI, GS, and GP) led to a reduction of the most undesirable variables, such as DS and HS. These results can be useful in future works, which will require a smaller number of test traits.

5. Conclusions

Of the 11 treatments included in the present study, the one that increased the desirable characteristics of physiological potential of the germination of genotypes was the gibberellic acid. This showed that dormancy on piquin pepper seed is mostly influenced by physiological aspects.

Genotypes 8 and 10, originating in Colatlán (Nursery), Ixhuatlán de Madero, Veracruz and La Laborcilla, Rioverde, and San Luis Potosí showed a greater germination capacity, as they were statistically superior in 5 of 6 evaluated variables.

The analysis of the main components through a biplot graph helps to understand in a practical way the interactions. The treatments had different effects on each genotype. G8, G2, and G10 genotypes showed higher germination speed when applying gibberellic acid and its combination with sanding and hydrochloric acid. Likewise, the interaction of the G8 and G10 genotypes with the Peroxide treatments of hydrogen + gibberellic acid and hydrochloric acid showed superiority in the germination index. In the case of the germination percentage, the combinations G2 and G7 with sanding + gibberellic acid and G8 without treatment stood out. Abnormal seedlings were reduced especially in the genotypes G1 and G3 by applying sanding + gibberellic acid and G9 without any treatment. A significant reduction of dead seeds in the G6 genotype was achieved by sanding and G7 with hydrogen peroxide + gibberellic

acid. The combination of G3 with hydrochloric acid and G9 with hydrogen peroxide + gibberellic acid and Agromil-V® greatly reduced hard seeds.

The relationship between the variables is an important factor that will allow us to predict a certain feature in future work.

The association between genotypes made it possible to know their similarity and diversity according to the characteristics studied.

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References

1. Medina-Martínez, T.; Villalón-Mendoza, H.; Pérez-Hernández, J.M.; Sánchez-Ramos, G.; Salinas-Hernández, S. Avances y perspectivas de investigación del chile piquín en Tamaulipas, México. *CienciaUAT* **2010**, *4*, 16–21.
2. González-Jara, P.; Moreno-Letelier, A.; Fraile, A.; Piñero, D.; García-Arenal, F. Impact of human management on the genetic variation of wild pepper, *Capsicum annuum* var. *glabriusculum*. *PLoS ONE* **2011**, *6*, e28715.
3. Cano-Vázquez, A.; López-Peralta, M.C.; Zavaleta-Mancera, H.A.; Cruz-Huerta, N.; Ramírez-Ramírez, I.; Gardea-Béjar, A.; González-Hernández, V.A. Variation in seed dormancy among accessions of chile piquín (*Capsicum annuum* var. *glabriusculum*). *Bot. Sci.* **2015**, *93*, 175–184.
4. Rueda-Puente, E.O.; Murillo-Amador, B.; Castellanos-Cervantes, T.; García-Hernández, J.L.; Tarazòn-Herrera, M.A.; Medina, S.M.; Barrera, L.E.G. Effects of plant growth promoting bacteria and mycorrhizal on *Capsicum annuum* L. var. *aviculare* ([Dierbach] D'Arcy and Eshbaugh) germination under stressing abiotic conditions. *Plant Physiol. Biochem.* **2010**, *48*, 724–730. [CrossRef] [PubMed]
5. Montes, H.S.; Ramírez, M.M.; Villalón, M.H.; Medina, M.T.; Morales, C.A.; Heredia, G.E.; Soto, R.J.M.; López, L.R.; Cardona, E.A.; Martínez, T.H.L. Conservación y aprovechamiento sostenible de chile silvestre (*Capsicum* spp. *Solanaceae*) en México. In *Avances Investigacion la Red Hortalizas del SINAREFI*; Libro Científico; INIFAP-CIR-CENTRO: Guanajuato, Mexico, 2006; pp. 71–134.
6. Pedraza, R.L.C.; Gómez, G.A.A. Análisis exploratorio del mercado y la comercialización de chile piquín (*C. annuum*, var. *aviculare Dierb.*) en México. *Tecsistecatl* **2008**, *1*, 5.
7. De la Rosa, M. Germination of simojovel pepper seeds (*Capsicum annuum* L.) previously exposed to NaCl and gibberellic acid. *Phyton (Buenos Aires)* **2012**, *81*, 165–168.
8. Votava, E.J.; Nabhan, G.P.; Bosland, P.W. Genetic diversity and similarity revealed via molecular analysis among and within an *in situ* population and ex situ accessions of chiltepín (*Capsicum annuum* var. *glabriusculum*). *Conserv. Genet.* **2002**, *3*, 123–129. [CrossRef]
9. Eshbaugh, W.H. Genetic and biochemical systematic studies of chili peppers (*Capsicum-Solanaceae*). *Bull. Torrey Bot. Club* **1975**, *102*, 396–403. [CrossRef]
10. Finch-Savage, W.E.; Leubner-Metzger, G. Seed dormancy and the control of germination. *New Phytol.* **2006**, *171*, 501–523. [CrossRef]
11. Finkelstein, R.; Reeves, W.; Ariizumi, T.; Steber, C. Molecular aspects of seed dormancy. *Annu. Rev. Plant Biol.* **2008**, *59*, 387–415. [CrossRef]
12. Doll, U.; Fredes, V.M.; Soto, V.C. Effect of different pre-germination treatments on germination of six native species from the mediterranean area of Chile. *IDESIA (Arica)* **2013**, *31*, 71–76. [CrossRef]

13. Prado-Urbina, G.; Lagunes-Espinoza, L.; García-López, E.; Bautista-Muñoz, C.; Camacho-Chiu, W.; Mirafuentes, G.F.; Aguilar-Rincón, V.H. Seed germination of wild chili peppers in response to pre-germination treatments. *Ecosistemas Recur. Agropecu.* **2015**, *2*, 139–149.
14. Félix-Herrán, J.A.; Sañudo-Torres, R.R.; Martínez-Ruiz, R.; Rojo-Martínez, G.E. Optimización del proceso germinativo de semillas de chile chiltepín [*Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill]. *Juyyaania* **2013**, *1*, 57–69.
15. Quintero, C.M.F.; Castillo, O.G.; Sánchez, P.D.; Marín-Sánchez, J.; Guzmán, A.I.; Sánchez, A.; Guzmán, J.M. Relieving dormancy and improving germination of Piquín chili pepper (*Capsicum annuum* var. *glabriusculum*) by priming techniques. *Cogent Food Agric.* **2018**, *4*, 1550275. [CrossRef]
16. García, F.A.; Montes, H.S.; Rangel, L.J.A.; García, M.E.; Mendoza, E.M. Physiological response of chili piquin [*Capsicum annuum* var. *glabriusculum* (Dunal) Heiser & Pickersgill] seeds to gibberlic acid and hot water. *Rev. Mex. Cienc. Agrícolas* **2010**, *1*, 203–216.
17. Asgari, A.; Moghaddam, P.R.; Koocheki, A. Methods for breaking Chinese lantern (*Physalis alkekengi* L.) seed dormancy. Laboratory and greenhouse studies. *Rev. Fac. Agron. LUZ* **2018**, *35*, 127–151.
18. González-Cortés, N.; Jiménez, V.R.; Guerra, B.E.C.; Silos, E.H.; de la Payro, C.E. Germination of amashito Chili (*Capsicum annuum* L. var. *Glabriusculum*) in southeastern Mexico. *Rev. Mex. Cienc. Agrícolas* **2015**, *6*, 2211–2218.
19. Koornneef, M.; Bentsink, L.; Hilhorst, H. Seed dormancy and germination. *Curr. Opin. Plant Biol.* **2002**, *5*, 33–36. [CrossRef]
20. Née, G.; Xiang, Y.; Soppe, W.J. The release of dormancy, a wake-up call for seeds to germinate. *Curr. Opin. Plant Biol.* **2017**, *35*, 8–14. [CrossRef]
21. Hayano-Kanashiro, C.; Gámez-Meza, N.; Medina-Juárez, L.Á. Wild pepper *Capsicum annuum* L. var. *glabriusculum*: Taxonomy, plant morphology, distribution, genetic diversity, genome sequencing, and phytochemical compounds. *Crop Sci.* **2016**, *56*, 1–11. [CrossRef]
22. Hernandez-Verdugo, S.; Guevara-González, R.G.; Rivera-Bustamante, R.F.; Oyama, K. Screening wild plants of *Capsicum annuum* for resistance to pepper huasteco virus (PHV): Presence of viral DNA and differentiation among populations. *Euphytica* **2001**, *122*, 31–36. [CrossRef]
23. Meyer, S.E.; Allen, P.S.; Beckstead, J. Seed germination regulation in *Bromus tectorum* (Poaceae) and its ecological significance. *Oikos* **1997**, *78*, 475–485. [CrossRef]
24. Hernández-Verdugo, S.; López-España, R.G.; Porras, F.; Parra-Terraza, S.; Villarreal-Romero, M.; Osuna-Enciso, T. Variation in germination among populations and plants of wild chili pepper. *Agrociencia* **2010**, *44*, 667–677.
25. Yan, W.; Tinker, N.A. Biplot analysis of multi-environment trial data: Principles and applications. *Can. J. Plant Sci.* **2006**, *86*, 623–645. [CrossRef]
26. Kendal, E.; Sener, O. Examination of genotype × environment interactions by GGE biplot analysis in spring durum wheat. *Indian J. Genet.* **2015**, *75*, 341–348. [CrossRef]
27. Letta, T.; D'Egidio, M.G.; Abinasa, M. Stability analysis of quality traits in durum wheat (*Triticum durum* Desf.) varieties under south Eastern Ethiopian conditions. *World J. Agric. Sci.* **2008**, *4*, 53–57.
28. Muthoni, J.; Shimelis, H.; Melis, R. Genotype x environment interaction and stability of potato tuber yield and bacterial wilt resistance in Kenya. *Am. J. Potato Res.* **2015**, *92*, 367–378. [CrossRef]
29. Haider, Z.; Akhter, M.; Mahmood, A.; Khan, R.A.R. Comparison of GGE biplot and AMMI analysis of multi-environment trial (MET) data to assess adaptability and stability of rice genotypes. *Afr. J. Agric. Res.* **2017**, *12*, 3542–3548.
30. Hagos, H.G.; Abay, F. AMMI and GGE biplot analysis of bread wheat genotypes in the northern part of Ethiopia. *J. Plant Breed. Genet.* **2013**, *1*, 12–18.
31. Gedif, M.; Yigzaw, D. Genotype by environment interaction analysis for tuber yield of potato (*Solanum tuberosum* L.) using a GGE biplot method in Amhara region, Ethiopia. *Agric. Sci.* **2014**, *5*, 239–249. [CrossRef]
32. Baraki, F.; Tsehaye, Y.; Abay, F. Analysis of genotype x environment interaction and seed yield stability of sesame in Northern Ethiopia. *J. Plant Breed. Crop Sci.* **2016**, *8*, 240–249.
33. Araya, E.; Gómez, L.; Hidalgo, N.; Valverde, R. Efecto de la Luz y del ácido gibérelico sobre la germinación in vitro de Jaul (*Alnus acuminata*). *Agron. Costarric.* **2000**, *24*, 75–80.
34. Karimpour, S.; Davarynejad, G.H.; Rouhbakhsh, H.; Ardakani, E. Data on scarification and stratification treatments on germination and seedling growth of *Ziziphus Jujuba* seeds. *Adv. Environ. Biol.* **2013**, *7*, 501–505.

35. Fredrick, C.; Muthuri, C.; Ngamau, K.; Sinclair, F. Provenance and pretreatment effect on seed germination of six provenances of *Faidherbia albida* (Delile) A. Chev. *Agrofor. Syst.* **2017**, *91*, 1007–1017. [CrossRef]
36. Batlla, D.; Benech-Arnold, R.L. A framework for the interpretation of temperature effects on dormancy and germination in seed populations showing dormancy. *Seed Sci. Res.* **2015**, *25*, 147–158. [CrossRef]
37. Nguyen, T.C.T.; Obermeier, C.; Friedt, W.; Abrams, S.R.; Snowdon, R.J. Disruption of germination and seedling development in *Brassica napus* by mutations causing severe seed hormonal imbalance. *Front. Plant Sci.* **2016**, *7*, 322. [CrossRef] [PubMed]
38. Liu, R.; Wang, L.; Tanveer, M.; Song, J. Seed heteromorphism: An important adaptation of halophytes for habitat heterogeneity. *Front. Plant Sci.* **2018**, *9*, 1515. [CrossRef] [PubMed]
39. Kucera, B.; Cohn, M.A.; Leubner-Metzger, G. Plant hormone interactions during seed dormancy release and germination. *Seed Sci. Res.* **2005**, *15*, 281–307. [CrossRef]
40. Rolston, M.P. Water impermeable seed dormancy. *Bot. Rev.* **1978**, *44*, 365–396. [CrossRef]
41. Rodríguez, L.Y.; Depestre, M.T.L.; Palloix, A. Behavior of new pepper (*Capsicum annuum* L.) F_1 hybrid and varieties with multirresistence to virus in open field conditions. *Cultiv. Trop.* **2014**, *35*, 51–59.